

What is claimed is:

1 1. A method of separating a sample material into a plurality of fractions,
2 comprising:
3 providing a system comprising:
4 a separation conduit having a separation matrix disposed therein and
5 a sample loading conduit in fluid communication with the separation conduit
6 at an intermediate point along the sample loading conduit;
7 bulk flowing a sample material into the sample loading conduit without substantially
8 displacing the separation matrix from the separation conduit;
9 injecting a portion of the sample material into the separation conduit; and
10 separating the sample material into a plurality of fractions.

1 2. The method of claim 1, wherein the sample loading conduit comprises a
2 loading end and a waste end, the loading end being contacted with a source of the sample material,
3 and further comprising applying a first pressure difference across the sample loading conduit to
4 move the sample material into the loading end of the sample loading channel and toward the waste
5 end of the sample loading channel.

1 3. The method of claim 1, wherein less than 10% of the separation matrix in the
2 separation conduit is displaced during the step of bulk flowing the sample material into the sample
3 loading conduit.

1 4. The method of claim 1, wherein less than 5% of the separation matrix in the
2 separation conduit is displaced during the step of bulk flowing the sample material into the sample
3 loading conduit.

1 5. The method of claim 1, wherein less than 1% of the separation matrix in the
2 separation conduit is displaced during the step of bulk flowing the sample material into the sample
3 loading conduit.

1 6. The method of claim 1, wherein the separation conduit is provided with a
2 higher flow resistance than the sample loading conduit.

1 7. The method of claim 6, wherein the separation conduit comprises one or
2 more of a greater length or a smaller cross-sectional area than the sample loading conduit.

1 8. The method of claim 1, wherein the sample loading conduit comprises a
2 loading end and a waste end, the loading end being contacted with a source of the sample material,
3 and further comprising applying a first pressure difference across the sample loading conduit to
4 move the sample material into the loading end of the sample loading channel and toward the waste
5 end of the sample loading channel.

1 9. The method of claim 8, wherein a negative pressure is applied to the waste
2 end of the sample loading conduit to supply the first pressure difference across the sample loading
3 conduit.

1 10. The method of claim 8, wherein the sample loading conduit and separation
2 conduit are in fluid communication at a first fluid junction, and further comprising moving a portion
3 of the sample material in the sample loading conduit through the first fluid junction and into the
4 separation conduit.

1 11. The method of claim 10, wherein the first fluid junction comprises a channel
2 segment connecting the sample loading conduit with the separation conduit.

1 12. The method of claim 10, wherein the step of moving the sample material
2 from the sample loading conduit through the fluid junction and into the separation conduit
3 comprises applying a voltage difference through the fluid junction to electrokinetically move the
4 portion of the sample material from the sample loading conduit into the separation conduit.

1 13. The method of claim 12, wherein the step of separating the sample material
2 comprises applying a voltage difference across the separation conduit, to electrophoretically
3 separate the sample material into different fractions.

20. The method of claim 1, wherein less than 75 % of the separation matrix is replaced in the replacing step.

21. The method of claim 1, wherein less than 50 % of the separation matrix is replaced in the replacing step.

22. The method of claim 1, wherein less than 20 % of the separation matrix is replaced in the replacing step.

23. The method of claim 1, wherein less than 10 % of the separation matrix is replaced in the replacing step.

24. The method of claim 1, wherein less than 5 % of the separation matrix is replaced in the replacing step.

25. The method of claim 1, wherein less than 1 % of the separation matrix is replaced in the replacing step.

26. The method of claim 1, wherein the separation conduit has at least one microscale cross-sectional dimension.

27. The method of claim 26, wherein the separation conduit is disposed in a microfluidic device.

28. The method of claim 1, further comprising:
providing a sample loading conduit fluidly coupled to the separation conduit at an intermediate point in the sample loading conduit, the sample loading conduit having a loading end and a waste end;

applying a first pressure difference across the sample loading conduit to move the second sample material into the loading end of the sample loading channel and toward the waste end of the sample loading channel; and

8 applying a second pressure difference across the separation conduit to move the
9 portion of the separation matrix out of the separation conduit toward the waste end of the sample
10 loading channel.

1 29. The method of claim 28, wherein a negative pressure is applied to the waste
2 end of the sample loading conduit, the negative pressure simultaneously supplying the first and
3 second pressure differences.

1 30. The method of claim 28, wherein the first and second pressure differences are
2 substantially the same, and wherein the separation conduit is dimensioned such that only the portion
3 of the separation matrix is removed from the separation conduit during the steps of applying the
4 first and second pressure differences.

1 31. The method of claim 28, wherein separation conduit is in fluid
2 communication with a source of separation matrix, and applying the second pressure difference
3 transports an amount of separation matrix into the separation conduit from the source of separation
4 matrix.

1 32. The method of claim 28, wherein the sample loading conduit and separation
2 conduit are in fluid communication at a first fluid junction, and further comprising moving a portion
3 of the second sample material in the sample loading conduit through the first fluid junction and into
4 the separation conduit.

1 33. The method of claim 32, wherein the step of moving the sample material
2 from the sample loading conduit through the fluid junction and into the separation conduit
3 comprises applying a voltage difference through the fluid junction to electrokinetically move the
4 portion of second sample material from the sample loading conduit into the separation conduit.

1 34. The method of claim 33, wherein the steps of separating the first and second
2 sample materials comprise applying a voltage difference across the separation conduit, to
3 electrophoretically separate each of the first and second sample materials into different fractions.

1 35. A method of separating a sample material into a plurality of fractions,
2 comprising:
3 providing a system that comprises:
4 a separation conduit having a separation matrix disposed therein;
5 a sample loading conduit in fluid communication with the separation conduit;
6 a source of sample material in fluid communication with the sample loading
7 conduit; and
8 a source of first reagent in fluid communication with the sample loading
9 conduit;
10 transporting the sample material and the first reagent into the sample loading
11 conduit, wherein the sample material and first reagent form a first mixture;
12 injecting a portion of the first mixture into the separation conduit; and
13 separating the sample material in the portion of the first mixture into a plurality of
14 fractions.

1 36. The method of claim 35, wherein the first reagent comprises a standard
2 marker compound, a labeling reagent, a diluent, or a detergent.

1 37. The method of claim 35, wherein the step of transporting the sample material
2 and first reagent into the sample loading conduit comprises applying a pressure difference across
3 the sample loading channel, the pressure difference moving an amount of sample material and an
4 amount of first reagent into the sample loading conduit from the source of sample material and the
5 source of first reagent, respectively.

1 38. The method of claim 37, wherein the source of first reagent is fluidly
2 connected to the sample loading conduit by a first reagent introduction channel, and wherein the
3 first reagent introduction channel and sample loading channel are dimensioned to transport a
4 selected ratio of sample material and first reagent into the sample loading conduit under the applied
5 pressure difference.

1 39. The method of claim 37, wherein the sample loading conduit comprises a
2 loading end and a waste end, and the pressure difference is applied by applying a negative pressure
3 to the waste end of the sample loading conduit.

1 40. The method of claim 1, wherein the step of injecting a portion of the mixture
2 into the separation conduit comprises applying a potential difference between the sample loading
3 conduit and the separation conduit to electrokinetically move the portion of the first mixture from
4 the sample loading conduit into the separation conduit.

1 41. A separation system, comprising:
2 a separation conduit having a first fluidic resistance and a flowable separation matrix
3 disposed therein;
4 a sample loading conduit fluidly connected to the separation conduit and having a
5 second fluidic resistance;
6 a sample loading system for transporting a sample material into the sample loading
7 conduit;
8 wherein the first fluidic resistance is higher than the second fluid resistance by an
9 amount sufficient to prevent substantial displacement of the separation matrix when sample material
10 is transported into the sample loading conduit.

1 42. A separation system, comprising:
2 a separation conduit having a flowable separation matrix disposed therein;
3 a sample loading conduit fluidly connected to the separation conduit;
4 a source of sample material in fluid communication with the sample loading conduit;
5 a source of a first reagent in fluid communication with the sample loading conduit by
6 a first reagent introduction channel;
7 a pressure or vacuum source coupled to the sample loading conduit for applying a
8 pressure difference across the sample loading conduit, wherein the sample loading conduit and first
9 reagent introduction channel are dimensioned to transport sample material and first reagent into the
10 sample loading conduit at a preselected ratio under the applied pressure difference.

1 43. A microfluidic device, comprising:

2 a body structure;
3 a sample loading channel disposed in the body structure;
4 a separation channel disposed within the body structure, the separation channel being
5 fluidly connected to the sample loading channel at a first fluid junction;
6 wherein the dimensions of the sample loading channel provide a lower fluid flow
7 resistance than the flow resistance provided by the dimensions of the separation channel.

1 44. The microfluidic device of claim 43, wherein the separation channel
2 comprises a flow resistance that is at least twice the resistance of the sample loading channel.

1 45. The microfluidic device of claim 43, wherein the sample loading channel
2 includes an external sampling pipettor element.

1 46. The microfluidic device of claim 43, wherein the first fluid junction is
2 disposed at an intermediate point in at least one of the sample loading channel and separation
3 channel.

1 47. The microfluidic device of claim 43, wherein the first fluid junction is
2 disposed at an intermediate point of each of the sample loading channel and the separation channel.

1 48. A method of separating sample materials, comprising:
2 providing a microfluidic device having a sample loading channel and a separation
3 channel, the separation channel being fluidly connected to the sample loading channel, and wherein
4 the separation channel comprises a separation matrix disposed therein;
5 bulk flowing a fluid sample material into the sample loading channel;
6 transporting a volume of the sample material from the sample loading channel into
7 the separation channel;
8 electrophoretically separating a sample material into separate fractions within the
9 separation channel;
10 replacing at least a portion of the separation matrix in the separation channel after
11 electrophoretically separating the sample material; and

12 repeating the bulk flowing, transporting and electrophoretically separating steps with
13 an additional sample material.

1 49. The method of claim 48, wherein substantially all of the separation matrix is
2 replaced during the replacing step.

1 50. The method of claim 48, wherein less than 90% of the separation matrix is
2 replaced I the replacing step.

1 51. The method of claim 48, wherein less than 50% of the separation matrix is
2 replaced.

1 52. The method of claim 48, wherein the bulk flowing, transporting,
2 electrophoretically separating, and replacing steps are repeated for each of at least two additional
3 sample materials.

1 53. The method of claim 48, wherein the replacing step is carried out during the
2 sample loading step.

1 54. The method of claim 53, wherein the sample loading step draws at least a
2 portion of the separation matrix out of the separation channel and into the sample loading channel,
3 and draws a volume of separation matrix into the separation channel from a separation matrix
4 reservoir that is in fluid the separation channel, thereby replacing the portion of the separation
5 matrix.